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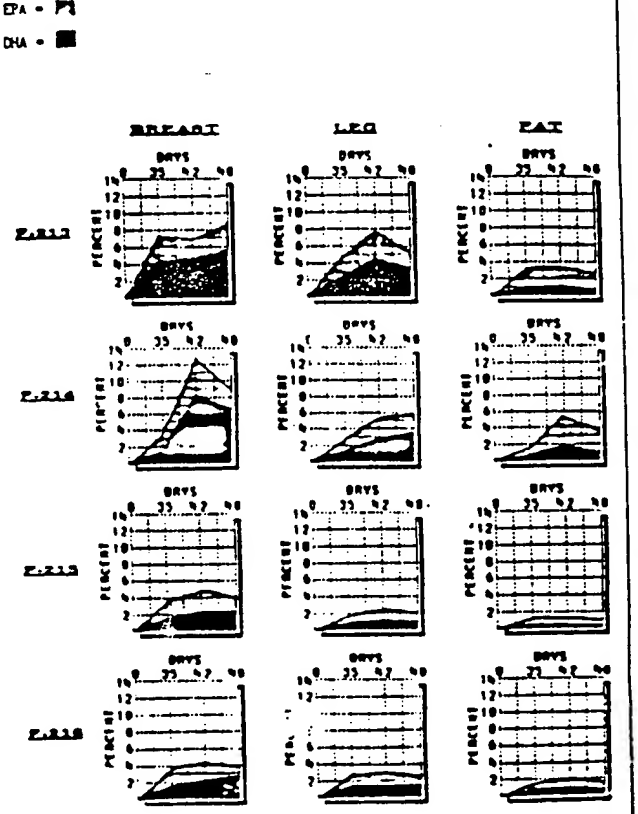
(57) Abstract

This invention provides a method of increasing the concentration of omega-3, polyunsaturated fatty acids in poultry which comprises administering to the poultry an effective amount of preformed omega-3, polyunsaturated fatty acid or a metabolic precursor thereof. The invention also involves a poultry feed composition useful in effecting this result. Also disclosed is a method of increasing the concentration of omega-3, polyunsaturated fatty acids in poultry eggs which comprises administering to the poultry egg layers an effective amount of preformed omega-3, polyunsaturated fatty acid or a metabolic precursor thereof. Further disclosed is a chicken and a poultry egg, each having omega-3, polyunsaturated fatty acids at a concentration greater than that which naturally occurs or is normally present.



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METHOD AND COMPOSITION FOR INCREASING THE CONCENTRATION OF OMEGA-3, POLYUNSATURATED FATTY ACIDS IN POULTRY AND POULTRY EGGS AND POULTRY AND EGGS RESULTING THEREFROM

Background of the Invention

Throughout this application, various publications are referenced by arabic numbers within parentheses. Full citations for these publications may be found at the end of the specification, immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by references into this application in order to describe more fully the state of the art to which this invention pertains.

Fish-eating communities (as in Denmark and Japan) have a markedly decreased incidence of coronary artery disease. Eskimo communities like-wise have a reduced incidence of coronary artery disease despite their heavy consumption of whale blubber (See generally, Refs. 1, 2, 3, 4, 5, 6 and 7). The mechanism of this reduced incidence of heart disease may only be secondarily correlated with a low serum cholesterol, but more importantly with a measurable tendency for decreased platelet adhesiveness (8, 9) and decreased whole blood viscosity (10). This, in turn, may be explained by the replacement in part, of arachidonic acid by omega-3 (n-3) polyunsaturated fatty acids (PUFA) in the cell membranes and the resultant changes in the functional properties of the prostaglandins derived from these.

It is theorized that dietary omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may provide one of the best

means of primary prevention of coronary artery disease through their effects on plasma lipids and platelet function. Of equal importance is the possible secondary prevention of progressive coronary artery atherosclerosis and peripheral vascular disease through similar mechanisms (i.e., cholesterol concentration, blood viscosity and platelet aggregability) (See, generally, Refs. 8-27) Omega-3 polyunsaturated fatty acids also may have a role in the treatment of specific illnesses (i.e. lupus, hypertension and immune problems) (See, e.g., Refs. 27, 28, 29, 30, 31, 32 and 33).

At this time, existing sources of dietary omega-3 PUFA are completely limited to fish and other marine animals (e.g., seals, whales), rare plants, and commercial extracts of whole fish as a liquid or encapsulated oil. (See, e.g., Refs. 34-41) Most land animals and vegetables have extremely low concentrations of eicosapentaenoic acid and docosahexaenoic acid. Furthermore, fish and other marine animals seem to be acceptable and easily available only to coastal fishing communities with a long history of fish as food. Most of the industrial land-locked communities find fish to be both too expensive and less appealing tastewise when compared to land animal meats. In addition, commercially available, highly refined fish oils are very expensive (refining of fish oil is necessary to limit potential toxic components such as vitamins A and D) (42). As daily dietary supplements, these fish oils lack taste appeal and are plagued by problems of user compliance.

In response to the above-mentioned shortcomings, this invention creates an alternative food which can provide a significant source of omega-3 PUFA without necessitating the consumption of fish or fish oils.

The experiments set forth herein establish a method of increasing the concentration of omega-3 PUFA in poultry and eggs for the purpose of creating a class of poultry and eggs with concentrations of omega-3 PUFA greater than that naturally occuring. The method involves administering to poultry an effective amount of either preformed omega-3 PUFA or a metabolic precursor thereof.

Summary of the Invention

This invention provides a method of increasing the concentration of omega-3, polyunsaturated fatty acids in poultry. The method comprises administering to the poultry an effective amount of preformed omega-3, polyunsaturated fatty acid or a metabolic precursor thereof.

Also disclosed is a poultry feed which comprises an amount of preformed omega-3, polyunsaturated fatty acid or a metabolic precursor thereof effective to increase the concentration of omega-3, polyunsaturated fatty acid in poultry which eat the feed.

The invention further discloses a chicken which comprises omega-3, polyunsaturated fatty acids at a concentration greater than that which naturally occurs or is normally present in poultry.

Additionally, this invention provides a method of increasing the concentration of omega-3, polyunsaturated fatty acids in poultry eggs. The method comprises administering to the poultry egg layers an effective amount of preformed omega-3, polyunsaturated fatty acid or a metabolic precursor thereof.

Finally, this invention discloses a poultry egg which comprises omega-3, polyunsaturated fatty acids at a concentration greater than that which normally occurs or is normally present in poultry eggs.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. EPA and DHA in Poultry Breast, Leg and Fat, Expressed as Percent of Total Lipids.

Figure 1 is a graphic representation of data from Table 1 and identifies feed compositions F-213, F-214, F-215 and F-216. Linolenic acid (Lin) values are not reflected in this figure. Omega-3 content values are only expressed for EPA and DHA. EPA and DHA are plotted additively, so that the combined percentage of these two substances can be observed.

Figure 2. EPA and DHA in Real World Poultry Breast and Leg, Expressed as Percent of Total Lipids.

Figure 2 reflects the omega-3 profile of the entire edible portion of breast and leg, both raw and cooked.

Detailed Description of the Invention

This invention provides a method of increasing the concentration of omega-3, polyunsaturated fatty acids in poultry. The method comprises administering to the poultry an effective amount of preformed omega-3 polunsaturated fatty acid or a metabolic precursor thereof.

Further disclosed is a poultry feed which comprises an amount of preformed omega-3, polyunsaturated fatty acid or a metabolic precursor thereof effective to increase the concentration of omega-3, polyunsaturated fatty acid in poultry which eat the feed.

Examples of the metabolic precursors which may be employed in this invention include linolenic acid, linseed oil, fish or a fish derivative, algae, and an omega-3 polyunsaturated fatty acid having a carbon chain of less than about 18 carbons.

This invention also discloses a chicken which comprises omega-3, polyunsaturated fatty acids at a concentration greater than that which naturally occurs or is normally present in poultry.

Additionally, this invention provides a method of increasing the concentration of omega-3, polyunsaturated fatty acids in poultry eggs. The method comprises administering to the poultry egg layers an effective amount of preformed omega-3, polyunsaturated fatty acid or a metabolic precursor thereof. The presently preferred metabolic precursor is menhaden oil and the presently preferred amount of menhaden oil comprises at least 5% by weight of the poultry's diet.

Finally, this invention discloses a poultry egg, which comprises omega-3, polyunsaturated fatty acids at a concentration greater than that which normally occurs or is normally present in poultry eggs.

Experimental Details

First Series of Experiments

METHOD

Step 1 - Experimental Feeding Trials

Several experimental feeding trials were tested as part of the omega-3 polyunsaturated fatty acid project design. Each trial was identified by a letter/number code. The trials varied with respect to the number of birds and pens employed, the type of feed treatments utilized, and the timing of the particular feeding programs.

Trial FR-26-86

The objective of this trial was to determine the EPA/DHA profile of carcass fat produced by various dietary regimes. The trial involved experimental feed treatments F-126 through F-129. (See Table 1). The feeding program was as follows:

0 - 21 Days = Common Starter (mixed-FR-24-86)
22 - 42 Days = Experimental Finisher
43 - 48 Days = Experimental Withdrawal

Four pens, with 100 birds per pen, were employed in this trial. The density of each pen was 0.80 sq. ft. per bird. All birds were banded according to their pen and diet. Body weights and feed conversions were collected at 21, 42 and 48 days of age. The birds were pulled from the processing line after being eviscerated, chilled, and then carried to the carcass lab.

Table 1

Feed Compositons: The following feed composition trials were used in this invention:

TRIAL #: FR-32-86

TMT		
F-164	Control (6% Poultry Meal)	
F-126	Corn	60.66%
	Soybean Meal	18.02%
	Gluten Meal	7.66%
	Poultry Meal	6.00%
	Meat-Bone Meal	4.00%
	Poultry Fat	2.08%
	Limestone	0.56%
	Salt	0.43%
	Premix	0.59%
F-165	Experimental (6% Poultry Meal + 2% Linseed Oil)	
F-127	Corn	60.66%
	Soybean Meal	18.02%
	Gluten Meal	7.66%
	Poultry Meal	6.00%
	Meat-Bone Meal	4.00%
	Linseed Oil	2.08%
	Limestone	0.56%
	Salt	0.43%
	Premix	0.59%
F-166	Experimental (10% Fish Meal)	
F-128	Corn	64.06%
	Soybean Meal	13.98%
	Fish Meal	10.00%
	Gluten Meal	8.54%
	Poultry Fat	1.94%
	Limestone	0.54%
	Salt	0.42%
	Premix	0.52%

TRIAL #: FR-32-86

TMT	Experimental (7% Fish Meal + 1% Linseed Oil)	
F-167		
F-129	Corn	63.18%
	Soybean Meal	13.86%
	Gluten Meal	10.56%
	Fish Meal	7.00%
	Meat-Bone Meal	2.18%
	Linseed Oil	1.00%
	Poultry Fat	0.76%
	Limestone	0.54%
	Salt	0.42%
	Premix	0.50%

TRAIL #: FR-41-86

TMT	Experimental (10% Menhaden Oil)	
F-213		
F-214	Corn	46.42%
	Soybean Meal	22.81%
	Menhaden Oil	10.00%
	Gluten Meal	8.60%
	Animal Blend	6.00%
	Brewex	2.00%
	Blood Meal	1.60%
	CDP	0.90%
	Limestone	0.68%
	Salt	0.31%
	Premix	0.68%

	Experimental (10% Linseed Oil)	
F-215		
F-216	Corn	46.42%
	Soybean Meal	22.81%
	Fish Meal	10.00%
	Gluten Meal	8.60%
	Animal Blend	6.00%
	Brewex	2.00%
	Blood Meal	1.60%
	CDP	0.90%
	Limestone	0.68%
	Salt	0.31%
	Premix	0.68%

Individual color scores were performed on all birds. Thereafter, the birds were frozen in storage facilities.

Trial FR-32-86

This project was designed to determine whether increasing dietary levels of fish meal or sources rich in linolenic acid (e.g., linseed oil) would produce broilers that contained high levels of omega-3, polyunsaturated fatty acids. The working hypothesis was that broilers consuming diets containing fish meal and/or linseed oil would contain higher levels of omega-3, polyunsaturated fatty acids than broilers fed the control diet containing 6% poultry meal and poultry fat.

The birds were fed experimental feed treatments F-164 through F-167 (see Table 1). The feeding program was set up as follows:

0 - 21 Days = Common Starter (mixed for FR-27-86)
22 - 48 Days = Experimental Finisher

Eight pens, with 90 male birds per pen, were employed in this trial. The density of each pen was 0.80 square feet per bird. Body weights and feed conversions at 21, 42 and 48 days of age. One hundred birds per treatment were collected, banded and then recovered from the processing line after eviscerating. The birds were iced down and transported to the carcass lab for individual color scores. After scoring, the birds were frozen and stored for taste panel work.

Taste panelists evaluated breast and thigh meat from FR-32-86 using the Hedonic Preference Evaluation. All

four treatments were included. First the panelists evaluated the breast meat as a group and then they tasted the dark meat. The meat was baked in the lab and warmed up in the microwave prior to serving. Overall (white and dark meat), there was a significant differences between F-165 and F-167, F-165 and F-166, F-164 and F-167, and F-164 and F-166. Looking at white meat only, there was no significant difference. However, with the dark meat, there was a significant difference between all the treatments. The panelists were influenced by whether or not the chicken meat was white or dark. Below is the data:

HEDONIC PREFERENCE EVALUATION

Smiley Score

	F-164	F-165	F-166	F-167
White Meat				
Average	3.29	3.31	3.43	3.34
Std. Dev.	1.43	1.43	1.42	1.37
Dark Meat				
Average	3.54	4.11	2.16	2.66
Std. Dev.	1.20	1.41	1.16	1.43

Preference:

White Meat: F-164 - 33%
F-166 - 31%
F-165 - 22%
F-167 - 14%

Dark Meat: F-165 - 55%
F-164 - 25%
F-167 - 14%
F-166 - 3%
none - 3%

General Comments:

(White Meat)

F-164 Taste - bland; good; aftertaste
Tenderness - tender
Moistness - dry slightly dry very dry
Texture - smooth; chewy

F-165 Taste - bland; good; okay
Tenderness - split between tough and tender
Moistness - dry slightly dry very dry
Texture - chewy; stringy; smooth; good

F-166 Taste - good; fishy; not tasty/funny
Tenderness - split between slightly tough and tender
Moistness - dry slightly dry moist very dry
Texture - smooth; chewy

F-167 Taste - bland; good; unfamiliar
Tenderness - very tender tender
Moistness - dry very dry slightly dry
Texture - good; smooth; stringy

(Dark Meat)

F-164 Taste - bland; okay; good
Tenderness - tender very tender
Moistness - moist; split between very moist, dry, and slightly dry
Texture - smooth; stringy; greasy; good

F-165 Taste - good; bland; okay
Tenderness - tender very tender
Moistness - moist; split between dry and very moist
Texture - smooth; good; soggy; greasy

F-166 Taste - awful, fishy; strange
Tenderness - tender
Moistness - split between dry and moist
Texture - smooth; greasy; stringy; good

F-167 Taste - awful; bland; fishy; strange; old
Tenderness - tender
Moistness - moist; split between slightly moist and dry
Texture - smooth; chewy; good

One breast half was cubed for the taste panel and the other half was sheared for tenderness. Overall, the breasts were tender. Below is the data:

Tenderness
(kgs./gms.)

	P-164	P-165	P-166	P-167
Average	4.31	3.48	4.97	3.76
Std. Dev.	1.41	0.58	1.68	1.30
No. _ 6.00	1	0	4	1
Range	3.01-7.65	2.22-4.27	3.05-7.17	1.89-6.45
No. Birds	10	10	11	11

Trial FR-45-86

This trial was designed to evaluate the effect on tissue omega-3 levels and the taste acceptance for broilers fed menhaden and linseed oils at several inclusion levels over various production time periods. Experimental feed treatments F-235 through F-252 (see Table 2) were used in this trial. The feeding program was as follows.

0 - 21 Days = Experimental Starter
22 - 43 Days = Experimental and/or Common Finisher
44 - 48 Days = Experimental or Common Withdrawal

Seventy-two pens, with 100 birds per pen, were employed. The density of each pen was 0.80 square feet per bird. Body weights and feed conversions were collected at 21, 43 and 48 days of age. On treatments F-236, F-239, F-242, F-245, F-248 and F-251, feeders were weighed and dumped only at 35 days. Two birds per pen (1 male and 1 female) were collected, the wings banded, and then each was processed at 3, 4, 5 and 6 weeks of age. This resulted in 4 males and 4 females per dietary treatment at each of the above ages. Of this group, one male and one female per treatment were shipped fresh on excess dry ice for fatty acid profiles. The remaining birds were frozen and stored for

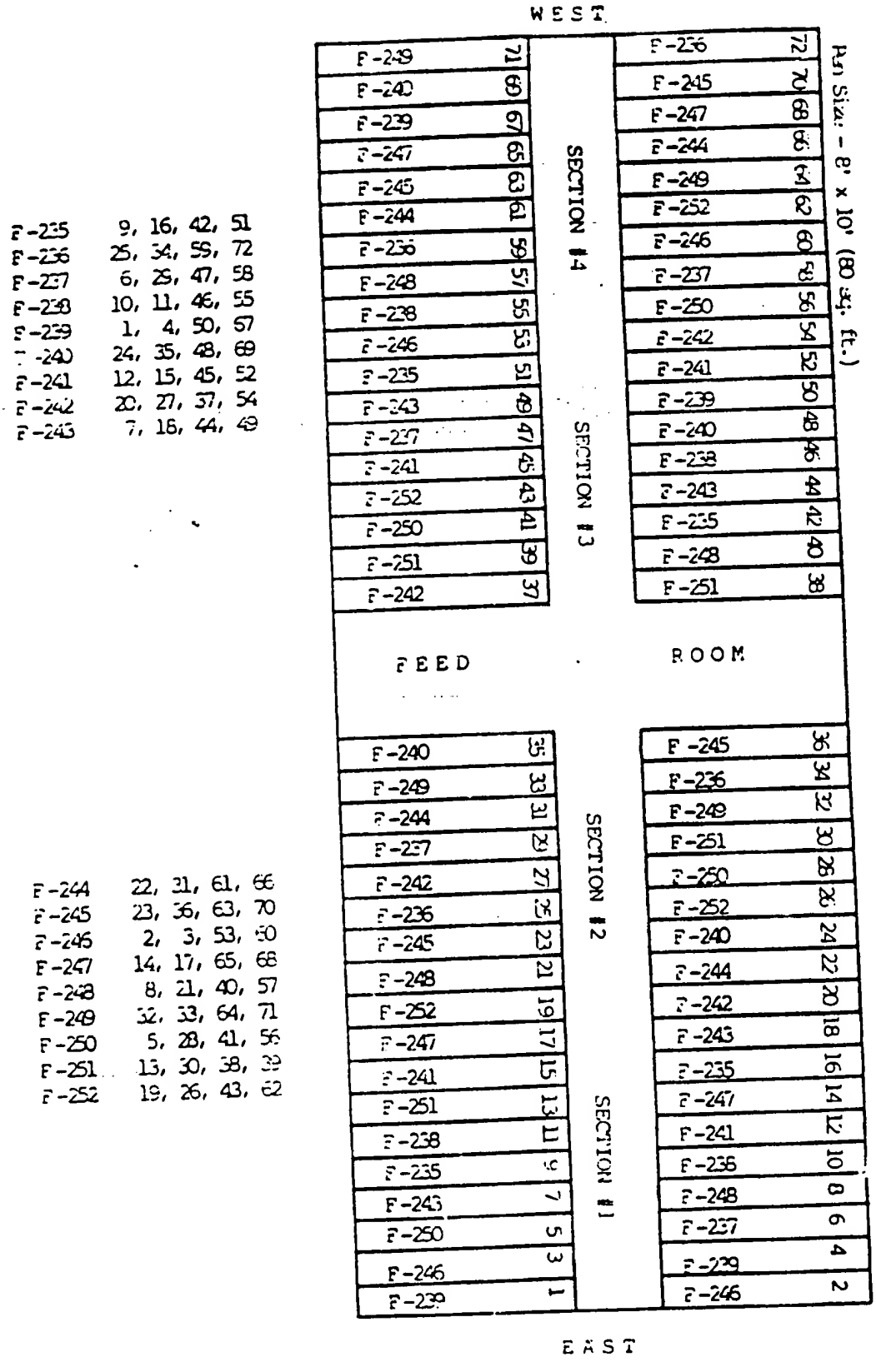
Table 2

Trial FR-45-86

EXPERIMENTAL TREATMENTS:

Source	Level	Experimental Feed Period	Common Feed Period
F-235 Menhaden Oil	2½%	Day 1 - Day 48	---
F-236 Menhaden Oil	2½%	Day 1 - Day 36	Day 37 - Day 48
F-237 Menhaden Oil	2½%	Day 1 - Day 21	Day 22 - Day 48
F-238 Menhaden Oil	5%	Day 1 - Day 48	---
F-239 Menhaden Oil	5%	Day 1 - Day 36	Day 37 - Day 48
F-240 Menhaden Oil	5%	Day 1 - Day 21	Day 22 - Day 48
F-241 Menhaden Oil	10%	Day 1 - Day 48	---
F-242 Menhaden Oil	10%	Day 1 - Day 36	Day 37 - Day 48
F-243 Menhaden Oil	10%	Day 1 - Day 21	Day 22 - Day 48
F-244 Linseed Oil	2½%	Day 1 - Day 48	---
F-245 Linseed Oil	2½%	Day 1 - Day 36	Day 37 - Day 48
F-246 Linseed Oil	2½%	Day 1 - Day 21	Day 22 - Day 48
F-247 Linseed Oil	5%	Day 1 - Day 48	---
F-248 Linseed Oil	5%	Day 1 - Day 36	Day 37 - Day 48
F-249 Linseed Oil	5%	Day 1 - Day 21	Day 22 - Day 48
F-250 Linseed Oil	10%	Day 1 - Day 48	---
F-251 Linseed Oil	10%	Day 1 - Day 36	Day 37 - Day 48
F-252 Linseed Oil	10%	Day 1 - Day 21	Day 22 - Day 48

ER-45-86



F-235	9, 16, 42, 51
F-236	25, 34, 59, 72
F-237	6, 29, 47, 58
F-238	10, 11, 46, 55
F-239	1, 4, 50, 67
F-240	24, 35, 48, 69
F-241	12, 15, 45, 52
F-242	20, 27, 37, 54
F-243	7, 18, 44, 49

F-244	22, 31, 61, 66
F-245	23, 36, 63, 70
F-246	2, 3, 53, 60
F-247	14, 17, 65, 68
F-248	8, 21, 40, 57
F-249	32, 33, 64, 71
F-250	5, 28, 41, 56
F-251	13, 30, 38, 39
F-252	19, 26, 43, 62

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future analysis. At 48 days of age, 40 birds per treatment (20 males and 20 females) were banded and processed; of this group one male and one female per treatment were collected and shipped on excess dry ice for fatty acid profiles. The remaining 38 birds per treatment were used in taste panel evaluation. The balance of all treatments were processed and scored for color and finish at 49 days of age.

Feeding Trial FR-41-86

This trial was designed to determine whether feeding extremely high levels of menhaden oil (10%) or linseed oil for one or two weeks during the grower phase would elevate tissue levels of omega-3 fatty acids in broilers processed at seven weeks of age. Consumer acceptance was evaluated in a taste panel study to determine whether such high levels of menhaden oil or linseed oil caused objectionable flavors in the final products.

The feed treatments employed in this trial were F-213 through F-216. (see Table 1) The experimental feed treatments and feeding program were as follows:

Experimental Treatments:

	Common Finisher	Common Grower	Experimental Grower	Common Withdrawal I
F-213	24-27 Days		Menhaden Oil 28-42 Days	42-48 Days
F-214	24-27 Days	28-34 Days	Menhaden Oil 35-42 Days	42-48 Days
F-215	24-27 Days		Linseed Oil 28-42 Days	42-48 Days
F-216	24-27 Days	28-34 Days	Linseed Oil 35-42 Days	42-48 Days

Feeding Program:

24 - 27 Days = Common Finisher
28 - 42 Days OR 35 -42 Days (See Above) = Experimental Grower
42 - 48 Days = Common Withdrawal I

Four pens, with 60 male birds per pen, were employed in this trial. The density of each pen was 0.80 square feet per pen. Two whole, processed male birds per treatment were collected at 28, 35, 42 and 48 days of age. Samples then were shipped with excess dry ice for fatty acid profiles. No bird or feed weighing was necessary. Feeders were dumped at all feed changes. The remaining birds were processed by treatment and frozen for later panel evaluation.

Step #2 Sample Preparation

Chemical preparation of tissue samples remained constant throughout the project. The term "chemical" refers to the extraction of crude lipids from the tissue sample and conversion to methyl esters (transesterification).

The following is a description delineating the evolution of sample handling.

Precision dissection

Whole birds were delivered to the laboratory under dry ice. The birds then were defrosted and grouped as per feed treatment. Using a scalpel, a core sample was removed from the breast (white meat), thigh (dark meat), fat , and skin. The term "core" is used to describe a sample derived by precision dissection, free of contamination by other tissue types (i.e. breast

tissue devoid of fat or skin). The quantity of tissue sample started out at 100gm for each of the four types. After establishing the representative concentration of crude lipids found in each tissue type, the sample size was adjusted to yield one gram of crude lipid after extraction. The resultant sample size was as follows:

1. Breast = 50gm
2. Leg = 25gm
3. Fat = 1 gm
4. Skin analysis was discontinued when it became obvious that it duplicated the information gathered from fat.

Real World Dissection

The term "Real World" refers to an attempt to duplicate a typical portion which would be eaten by the consumer. The following is a description of each "Real World" type:

"REAL WORLD BREAST" was comprised of all edible tissue referred to as breast by the consumer. This included muscle, skin, and fat.

"REAL WORLD LEG" contained all edible tissue found in the thigh and leg, including muscle, skin, and fat.

Depot Fat Dissection

After reviewing the initial data, the decision was made to focus efforts on increasing omega-3 PUFA concentration in fat (including leaf fat), hereinafter called depot fat. The other tissue types (core, breast and

leg) being more metabolically active, are closer to blood levels of omega-3 PUFA, give consistently higher values, but realistically contribute less to real world sample levels of omega-3 PUFA than depot fat. The harvesting of depot fat is hereinafter referred to as depot fat dissection.

Current Dissection Technique

"Depot Fat Dissection" was employed at the ages of 21, 28, 35 and 42 days. At 49 days, when the animal is normally prepared for consumer use, whole birds were received and tested using "Real World Dissection".

Step # 3 Analysis of Long Chain Fatty Acids in Foods and Blood : Brief Summary

The sample was homogenized with chloroform:methanol 2:1 to quantitatively extract the total crude lipids. The fatty acids from triacylglycerides, phosphatides and cholesterol esters were converted into methyl esters by a transesterification reaction using sodium methoxide. The resulting methyl esters were then analyzed by capillary gas chromatography and mass spectrophotometry (Perkin-Elmer/Finnigan Mass Spectrophotometer - Ion Trap).

Step # 4: Extraction:

Methanol was added to the weighed sample in step #1, in a homogenizing vessel of appropriate size, and in a volume representing 10X the sample weight (10ml methanol/gram of sample). The sample was homogenized for one minute, taking care to avoid excessive heat generation. Added next was a volume of chloroform which was

2X the amount of methanol added previously (20ml chloroform / gram of sample). The sample was homogenized once again for 2 minutes. Then, the sample was centrifuged and the supernatant was filtered into a suction flask through Whattman #1 filter paper in a buchner funnel. Celite analytical filtering aid was used, if necessary, to promote fascer flow.

The filtrates were then transfered quantitatively into a separatory funnel of appropriate volume. A small portion of chloroform:methanol 2:1 was used to rinse the suction flask and to insure quantitative transfer of extract into the separatory funnel.

A volume of 0.88% potassium chloride in water, equal to 25% the volume of organic extract, was next added to the separatory funnel. The mixture was shaken vigorously, then allowed to settle. When the phase separation was complete (both layers were clear and no emulsion existed at the interface), the bottom organic layer was drained off into a second clean separatory funnel of the same size and was washed with a mixture of water:methanol 1:1, the volume of which was equal to 25% that of the organic layer. After complete phase separation, the bottom organic layer, which contained the purified lipids, again was drained off into an erlenmeyer flask of appropriate volume and fitted with a ground glass stopper. Two grams of anhydrous sodium sulfate were added and the flask was shaken to dry the extract. The flask was then swirled to rinse the sodium sulfate down to the bottom and the solution was decanted into a round bottom flask of appropriate volume, with care taken to leave the soduum sulfate behind.

Step # 5 Concentration and Isolation :

A round bottom flask was connected to a rotory evaporator using a trap and the solvent was removed at or near room temperature and under reduced pressure. (temperature may be up to 40 degrees centigrade). The solvent was not evaporated to dryness, but rather concentrated to a small volume (about 25 ml). The extract was quantitatively transferred from the round bottom flask into a tared 25 X 150 mm test tube, using a small portion of chloroform:methanol 2:1 to rinse the flask. The tube was placed in a heating block calibrated to 40 degrees centigrade and the extract was evaporated to dryness using a gentle stream of nitrogen. The tube was weighed, subtracting the tare weight, and the weight of total crude lipid present was calculated. This value then was recorded for future reference.

Immediately after weighing the extract, the lipid was redissolved in petroleum ether to a concentration of 30 mg/ml. The headspace was flushed with nitrogen and the tube stoppered.

At the end of this step, a solution of crude lipid extract in petroleum ether was prepared in a volumetric flask, at a concentration at or near 25 mg/ml. The concentration then was recorded. Then, 1.0 ml of this solution was transferred into a 15 ml teflon lined screw-capped vial and 1.0 ml of methanoic base reagent was added, mixed and stoppered tightly.

Step # 6 Transesterification :

The vial was heated at 80 degrees centigrade for 20 minutes in a heating block and allowed to cool to room temperature. Following this, 3 ml of water and 3 ml of diethylether were added to the vial and mixed well. After complete phase separation had occured, the lower aqueous layer was removed, using a pasteur pipet, and discarded. The organic layer (petroleum ether/diethylether/fatty acid methyl ester solution) was washed once more with 3 ml of water. Once again, the aqueous layer was discarded and removed. A small amount of anhydrous sodium sulfate then was added to the test tube. The tube was shaken to dry the contents over sodium sulfate and the contents then were transferred quantitatively into a 5 ml reaction vial using a pasteur pipet. The volume was adjusted to exactly 4.0 ml either by exaporation with nitrogen or by adding petroleum ether. The vial was stoppered tightly with a mininert valve and stored in the freezer until ready for gas chromatograph analysis.

Step # 7 Gas Chromatographic Analysis :

From the vial prepared in step # 4, 0.1 microliters were withdrawn and injected into a Perkin-Elmer Sigma 2000 Gas Chromatograph equipped with a microprocessor to control four level temperature programming, flame ionization detector and capillary injector for split/splitness operation and using the following conditions:

Injection Temperature	250 degrees centrigrade
Split Mode Ration	100 : 1

Column Temperature Program	150 to 220 at 2 degrees centigrade / minute
Attenuation	4 to 16 FID
Detector Temperature	250 degrees centigrade
Carrier Flow Rate	0.7 - 1.0 ml / minute
	helium

The resulting chromatogram was observed and the parameters adjusted for optimum sensitivity and resolution. When necessary, the sample was diluted or concentrated.

Discussion

Table 3 represents eicosapentaenoic acid, docosahexanenoic acid and linolenic acid values in poultry breast, leg and fat. These values are expressed as a percentage of total lipids.

The areas in table 3 which are crossbatched were designed to be experimental controls; however, chromatographic analysis indicates that these control animals have been fed feed containing significant amounts of linolenic acid; resulting in the expected abnormal quantities of EPA and DHA (metabolic conversion).

Table 3 illustrates the following:

Ba, La, & Fa = Breast, Leg, and Fat at 35 days. (precision disection)

Bb, Lb, & Fb = Breast, Leg, and Fat at 42 days. (precision disection)

Bc, Lc, & Fc = Breast, Leg, and Fat at 48 days. (precision disection)

Table 3

Quantitative Values for LIN, EPA and DHA Long Chain Fatty Acids In Poultry Breast, Leg and Fat

	Ba	La	Fa	Bb	Lb	Fb	Bc	Lc	Fc	Brwr	Lrwr	Brwc	Lrwc
213 LIN	0.77	1.02	1.00	0.74	0.94	1.18	0.60	0.68	0.91	0.94	0.97	0.96	0.97
213 EPA	3.43	2.40	2.14	2.52	3.32	2.46	2.58	1.79	1.88	2.32	1.88	1.95	1.90
213 DHA	4.33	2.23	0.90	4.37	4.40	1.09	5.89	3.38	0.67	1.69	1.21	1.79	1.47

	Ba	La	Fa	Bb	Lb	Fb	Bc	Lc	Fc	Brwr	Lrwr	Brwc	Lrwc
214 LIN	2.91	4.54	5.19	0.79	1.05	1.13	0.90	0.85	1.11	1.03	1.04	0.84	1.01
214 EPA	1.73	1.19	0.92	4.44	2.41	3.57	2.18	2.10	2.49	2.07	2.17	1.87	1.91
214 DHA	1.84	1.45	0.58	8.11	2.76	1.96	6.62	3.67	1.02	1.60	1.56	1.75	1.27

Table 3 (cont.)

Quantitative Values for LIN, EPA and DHA Long Chain Fatty Acids In Poultry Breast, Leg and Fat

	Ba	La	Fa	Bb	Lb	Fb	Bc	Lc	Fc	Brwr	Lrwr	Brwc	Lrwc
215 LIN	7.74	11.74	13.09	8.21	13.49	14.80	7.13	10.55	12.16	10.98	11.85	8.66	9.60
215 EPA	1.78	0.86	0.68	2.53	1.41	0.75	1.86	0.92	0.66	0.71	0.64	0.79	0.65
215 DHA	1.77	0.80	0.36	2.16	1.28	0.26	2.12	0.98	0.28	0.50	0.35	0.61	0.54

	Ba	La	Fa	Bb	Lb	Fb	Bc	Lc	Fc	Brwr	Lrwr	Brwc	Lrwc
216 LIN	2.91	4.54	5.19	5.13	7.16	9.02	2.94	4.10	5.30	4.98	5.57	5.46	5.77
216 EPA	1.73	1.19	0.92	2.01	1.45	1.13	1.16	0.93	0.8	0.78	0.79	0.89	0.76
216 DHA	1.84	1.45	0.92	2.14	1.42	0.82	2.74	1.57	0.48	0.51	0.47	0.75	0.58

Brwr & Lrwr = Breast, Leg, and Fat at 48 days. (real world raw disection)

Brwc & Lrwc = Breast, Leg, and Fat at 48 days. (real world cooked disection)

As is shown in Figure 1, sample feed compositions F-213 and F-214 demonstrated an ability to generate 2% EPA in all three tissue types. DHA varied from 0.7% to over 6%, with the greatest values found in breast tissue. Furthermore, omega-3 increased rapidly with this feeding technique. Figure 1 also shows that sample feed compositions F-215 and F-216 demonstrated a significant ability to generate metabolically EPA and DHA using the metabolic precursor linolenic acid in the feed.

As is evidenced in Figure 2, "Real world" samples demonstrated the importance of elevating omega-3 levels in depot and subcutaneous fat. "Precision" tissue type disection revealed a lipid content of Breast = 1%, Leg = 2%; while "real world" disection yielded a lipid content of Breast = 7-1/2% and Leg = 15%.

Figure 2 illustrates the heat stability of EPA and DHA. Cooking tests conducted with fish have yielded similar results.

Second Series of Experiments

Method

Each egg was separated grossly into yolk and white. The white then was discarded. The egg yolk was analyzed using the same analysis employed and previously described in the first series of experiments.

Discussion

The "MARK" EGG" data charted in Table 4 resulted from an experiment designed to establish the level of naturally occurring n-3 PUFA found in eggs available to consumers.

The "Omega Egg" data charted on Table 5 reflects the n-3, PUFA levels obtained in eggs from hens fed on an experimental diet containing preformed and/or a metabolic precursor of n-3 PUFA. The source of the latter in this experiment was menhaden oil at a concentration of 5% by weight of the feed. Eggs were tested at weeks 1 thru 10. Organoleptic scoring of a taste panel indicated excellent taste and appeal.

Table 4

"MARKET EGG" n-3 PUFA PROFILE
(EXPRESSED AS PERCENT OF CRUDE LIPID)

Linolenic Acid	0.16
Eicosapentaenoic Acid	0.01
Docosahexaenoic Acid	0.58

Table 5

"OMEGA EGG" n-3 PUFA PROFILE
(EXPRESSED AS PERCENT OF CRUDE LIPID)

Weeks of Experimental Diet	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Linolenic Acid	0.20	0.42	0.44	0.42	0.41	0.31	0.30	0.36	0.46	0.42
Eicosapentaenoic Acid	0.11	0.64	0.82	0.74	0.84	0.39	0.64	0.59	0.63	0.53
Docosahexaenoic Acid	0.88	3.12	3.20	3.14	3.62	2.64	2.82	2.57	2.89	2.67

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What is claimed is:

1. A method of increasing the concentration of omega-3, polyunsaturated fatty acids in poultry which comprises administering to the poultry an effective amount of preformed omega-3, polyunsaturated fatty acid or a metabolic precursor thereof.

2. A method of claim 1, wherein the metabolic precursor comprises linolenic acid.

3. A method of claim 2, wherein the linolenic acid is present in the form of linseed oil.

4. A method of claim 1, wherein the metabolic precursor comprises fish or a fish derivative.

5. A method of claim 1, wherein the metabolic precursor is derived from algae.

6. A method of claim 1, wherein the metabolic precursor comprises an omega-3, polyunsaturated fatty acid having a carbon chain of less than about 18 carbons.

7. A poultry feed which comprises an amount of preformed omega-3, polyunsaturated fatty acid or a metabolic precursor thereof effective to increase the concentration of omega-3, polyunsaturated fatty acid in poultry which eat the feed.

8. A poultry feed of claim 7, wherein the metabolic precursor comprises linolenic acid.

9. A poultry feed of claim 8, wherein the linolenic acid is present in the form of linseed oil.

10. A poultry feed of claim 7, wherein the metabolic precursor comprises fish or a fish derivative.

11. A poultry feed of claim 7, wherein the metabolic precursor is derived from algae.

12. A poultry feed of claim 7, wherein the metabolic precursor comprises an omega-3, polyunsaturated fatty acid having a carbon chain of less than about 18 carbons.

13. A chicken which comprises omega-3, polyunsaturated fatty acids at a concentration greater than that which naturally occurs or is normally present in poultry.

14. A method of increasing the concentration of omega-3, polyunsaturated fatty acids in poultry eggs comprising administering to the poultry egg layers an effective amount of preformed omega-3, polyunsaturated fatty acid or a metabolic precursor thereof.

15. The method of claim 14, wherein the metabolic precursor comprises menhaden oil.

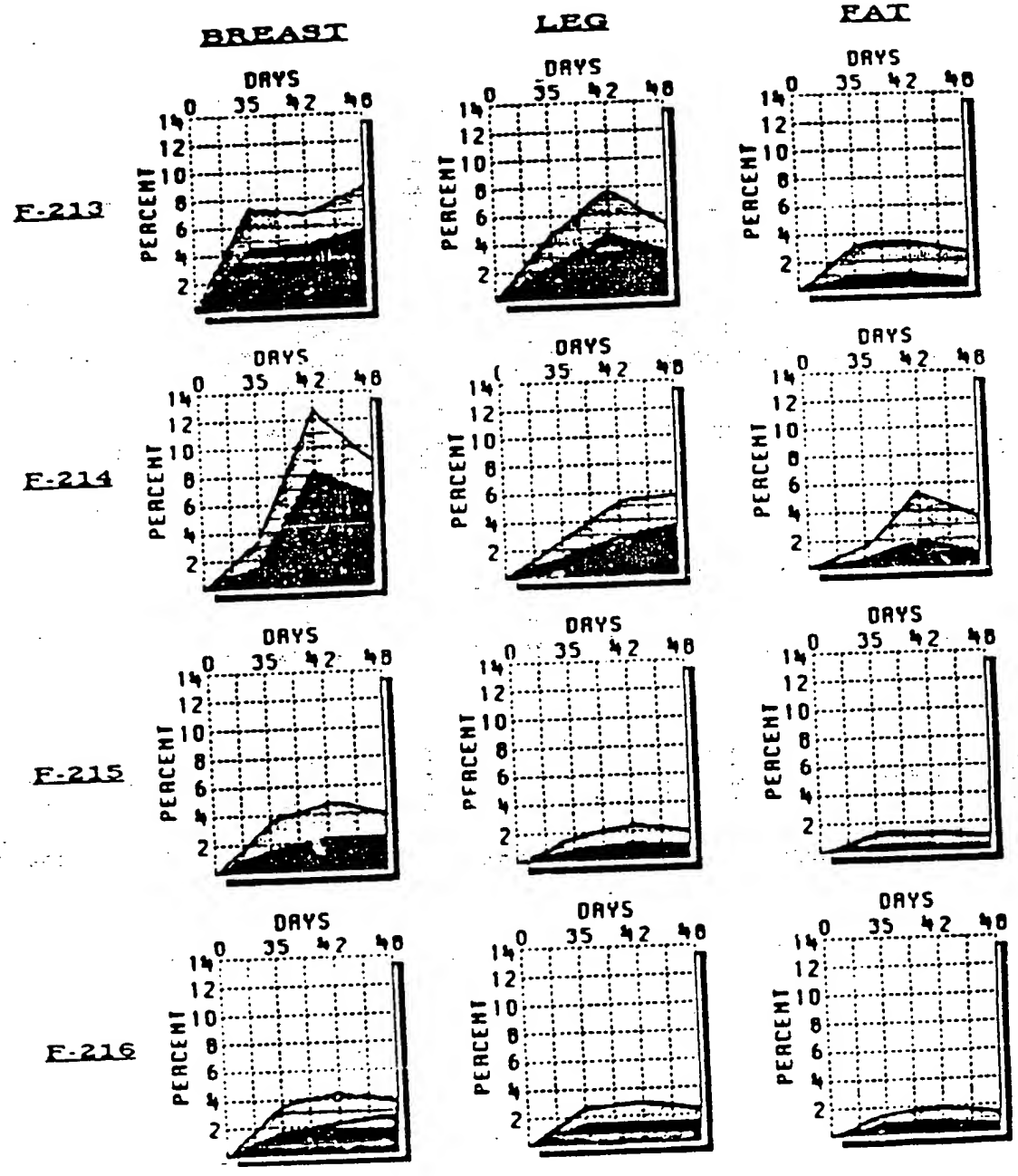
16. The method of claim 15, wherein the menhaden oil comprises at least 5% by weight of the poultry egg layers diet.

17. A poultry egg which comprises omega-3, polyunsaturated fatty acids at a concentration greater than that which normally occurs or is normally present in poultry eggs.

Figure 1

EPA = ▭

DHA = ■

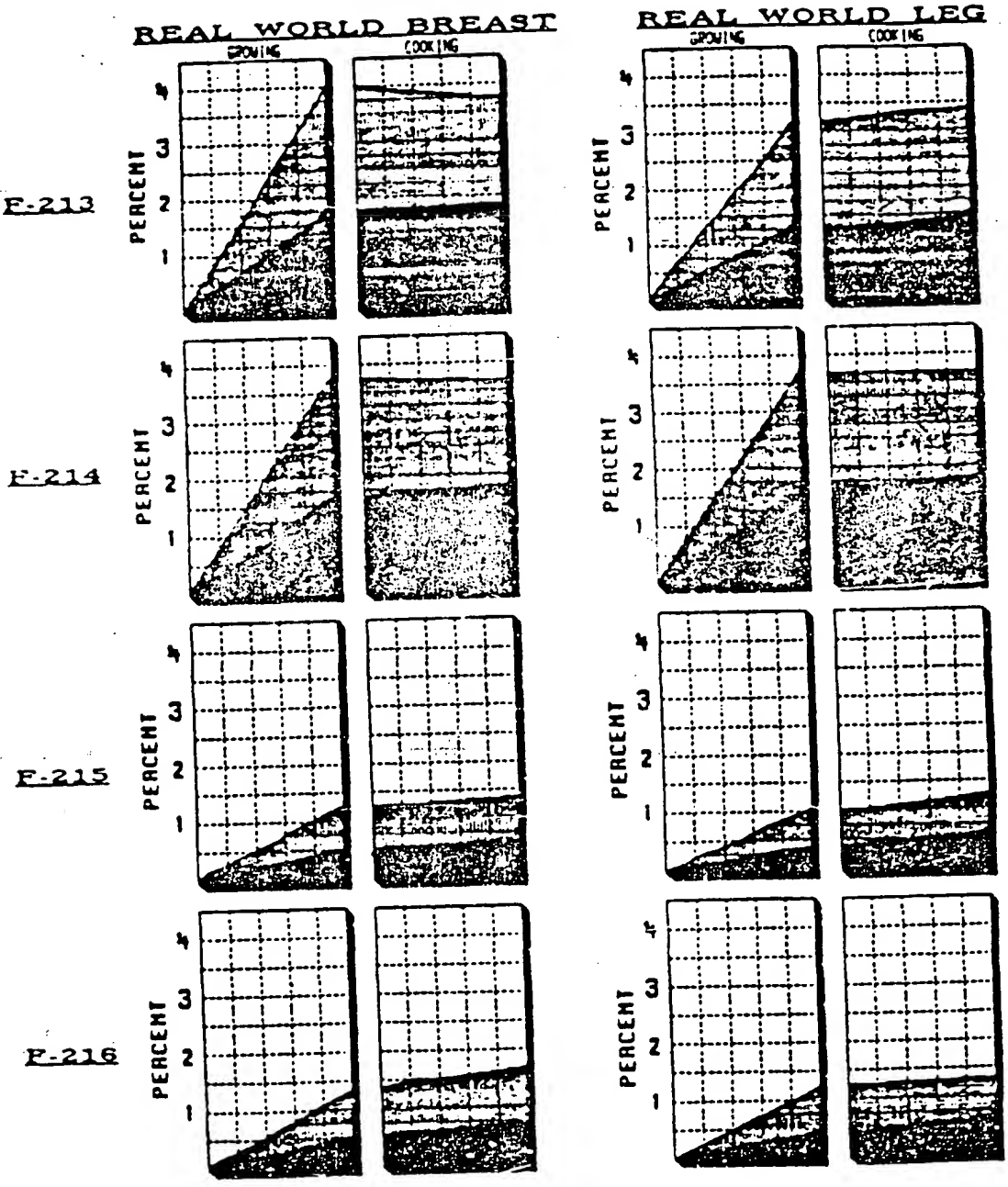


2/2

Figure 2

EPA = ▒

DHA = ■



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/02048

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): A61K 31/20

U.S.Cl.: 514/560

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	514/560

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X/Y	U.S., A, 2,879,162 (BALDINI ET AL) 24 March 1959. See the entire document.	1,2,4, 6-12,14 / 3,5,15,16
X/Y	JP, B, 61-216658 (AGENCY OF IND SCI TECH (NOSA KANF)) 26 September 1986. See the Derwent Abstract attached thereto.	1,2,4, 6-12,14 / 3,5,15,16
X	JP, B, 57-86254 (NISSUI SEIYAKU KK (NIUS)) 29 May 1982. See the Derwent Abstract attached thereto.	1,2,4, 6-12,14
X	JP, B, 60-132916 (NISSHIN OIL MILLS KK) 16 July 1985. See the Derwent Abstract attached thereto.	1.2,4, 6-12,14
Y	JP, B, 60-169418 (NIPPON OILS & FATS KK) 02 September 1985. See the Derwent Abstract attached thereto.	3,5,15, 16

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

06 SEPTEMBER 1988

Date of Mailing of this International Search Report

19 OCT 1988

International Searching Authority

ISA/US

Signature of Authorized Officer

FREDERICK E. WADDELL

Form PCT/ISA/210 (second sheet) (Rev 11-87)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1 ☐ Claim numbers because they relate to subject matter [2] not required to be searched by this Authority, namely:

2 ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3 ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1-12 and 14-16 drawn to a method and composition for treating poultry; class 514 subclass 560.

II. Claims 13 and 17 drawn to a chicken and an egg classified in class 800 subclass 1.

1 ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2 ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3 ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1-12 and 14-16

4 ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.